THE VETERINARY RECORD

FOUNDED BY WILLIAM HUNTING, F.R.C.V.S., 1888

No. 42. OCTOBER 18, 1952 VOL. 64.

EXPERIMENTS WITH AN AVIANISED STRAIN OF THE ORGANISM OF CONTAGIOUS BOVINE PLEUROPNEUMONIA

BY

D. SHERIFF

AND

S. E. PIERCY,

VETERINARY RESEARCH LABORATORY, KABETE, KENYA

Vaccination against contagious bovine pleuropneumonia (C.B.P.P.) in East Africa is based on the use of "culture vaccine" (Walker, 1919-22) which consists of a strain of the organism attenuated by serial culture over a varying number of generations in 10 per cent. bovine serum broth (B.S.B.). An initial course of three doses at intervals of three weeks is given, followed by a single yearly dose. Local reactions and the level of immunity following vaccination are variable. Furthermore, it is difficult adequately to test batches of vaccine within the limits of time imposed by its poor keeping qualities. In recent years, the attitude of African cattle owners has made it necessary to use a vaccine with a wide margin of safety and in consequence its immunising value has become insufficient to do more than reduce mortality. It is our opinion that weak vaccines of this type tend to increase the number of "carriers" and render control more difficult.

For these reasons the vaccine is of limited value and experiments were, therefore, carried out to determine the effect of growing the C.B.P.P. organism in embryonated hens' eggs in an attempt to overcome some or all of these drawbacks.

MATERIALS AND METHODS

(1) STRAIN.—Strain T1: this was obtained from an ox (1236) inoculated subcutaneously on January 27th, 1951, with pleuritic exudate ("lymph") from a natural case of C.B.P.P. in Tanganyika. Bovine 1236 was killed *in extremis* on February 13th, 1951, and a pure culture in B.S.B. obtained from the lesion. Another animal (1232), inoculated with the same material, developed a swelling 6 inches in diameter but recovered. Exudate ("tumour lymph") from the subcutaneous lesion of No. 1236, after storage at room temperature for six and eight weeks respectively, was given subcutaneously to bovines 1205 and 1604. The former reacted and recovered; the latter did not react. Three other animals were given second generation culture in B.S.B. Two of these did not react, the third developed a small swelling which resolved. There is a possibility, therefore, that strain T1 was initially comparatively avirulent though the prolonged storage of the "lymph" may have been responsible for its apparent lack of potency.

(2) TECHNIQUE.—The general procedure used for chick-embryo culture was that described by Piercy (1952). Eight- or nine-day-old eggs were normally used. Membrane, embryo and allantoic fluid transfers were made with undiluted material except in the titration experiments. On some occasions 250 units of penicillin per c.c. were used to control contamination. Lyophilisation was carried out on an Edwards freeze-drier* and ampoules were sealed *in vacuo*.

(3) ANIMAL INOCULATIONS.—Cattle were inoculated subcutaneously about 6 inches behind the shoulder; in this position local reactions are readily assessed. The animals ranged from high grade to pure native cattle, usually 18 to 30 months old, but some calves and old animals were included.

(4) CHALLENGES.—The immunity of inoculated cattle was tested by the subcutaneous inoculation of 1·0 c.c. of "lymph" or "tumour lymph" on the side of the chest opposite to that used for the original inoculation.

(5) COMPLEMENT FIXATION TESTS.—Antibodies were demonstrated by the complement fixation test based on the technique of Campbell and Turner (1936) and using antigen supplied through the courtesy of the Animal Health Laboratory, Melbourne, Australia. Results are recorded thus:—

4/10=complete fixation; no haemolysis at a serum dilution of 1:10.

3/10 and 2/10=incomplete fixation; increasing amounts of haemolysis.

1/10=haemolysis almost complete.

−ve=haemolysis complete.

Sera were diluted 1:10 and thereafter in twofold dilutions.

GROWTH IN EMBRYONATED EGGS

SERIES I

Twenty-four nine-day-old eggs were inoculated on to the chorio-allantoic membrane with a fresh first generation culture of strain T1 in B.S.B. and incubated for four days at 35° C. When harvested, a number of membranes from live eggs were found to be noticeably thickened and oedematous round the site of inoculation. A pure culture of C.B.P.P. organisms was obtained in B.S.B. from the chorio-allantoic membrane, live embryos and allantoic and amniotic fluids. Serial transfers, using the same technique except that incubation was carried out at 37° C. after the first eight passages, were made over 27 egg passages, harvesting usually on the fourth or fifth day. Gross white thickening and oedema of the chorio-allantoic membrane occurred at an early stage in the series, frequently involving the whole membrane. Definite isolated

* W. Edwards & Co. (London) Ltd.

focal lesions, characteristic of many egg-adapted viruses, were not, however, observed.

The effect of the organism on the embryo was not well demonstrated in this series because frequently harvesting was carried out on the fourth day. Since the main object of the work at this stage was to pass the organism rapidly through a number of generations to observe whether any modification for cattle took place, the effect on the embryo of allowing longer periods of incubation was not regularly estimated. But on those occasions when incubation was permitted for longer periods, deaths were common on the fifth day.

Series II

A yolk sac inoculation series was commenced after 10 membrane passages. Using chorio-allantoic membrane harvested from the 10th passage (Series I), 16 eggs were inoculated into the yolk sac and incubated for eight days. The more lethal effect on the embryo using this route of inoculation was at once apparent, deaths occurring most commonly on the fourth, fifth and sixth days after inoculation. Checks on dead embryos for the presence of C.B.P.P. organisms and the absence of bacterial contaminants were made on several occasions with satisfactory results.

This series was continued up to the 35th passage. Owing to unavoidable circumstances, the 20th to the 23rd passage had to be continued with membrane to membrane inoculations.

Series III

A third series was started using membranes of the 25th passage (Series I) which had been dried and stored at −20° C. for four months. This was done because on three occasions in Series II (passages 25, 28 and 31) contamination occurred, necessitating the use of serum broth cultures, which had been inoculated to demonstrate the presence of C.B.P.P. organisms, to continue the series. Coincidentally, unexpected results, to be discussed later, occurred in cattle which received egg material from these passages. It was considered advisable, therefore, to return to material which had an unbroken history of egg to egg passage. This series is now in its 42nd passage, using allantoic fluid for serial transfers and harvesting dead embryos on the third or fourth day for animal inoculations. Deaths occur regularly on the third and fourth days. Deaths on the second day are probably mainly non-specific but on one occasion at least (generation 41) organisms were present in the embryo in sufficient numbers to cause the usual mortality in the next generation.

Titration Experiments

Using dead embryos of the 17th generation (Series II), tenfold dilutions in saline were made. A tube of serum broth, and six eggs (into the yolk sac) were inoculated from each dilution. Growth occurred in all the serum broth tubes (highest dilution of inoculum 10^{-7}). Deaths occurred, and organisms were recovered from dead embryos in all six lots of eggs (highest dilution of inoculum 10^{-6}). This titration was repeated with the next generation and very similar results were obtained.

A third titration was carried out using chorio-allantoic membranes harvested from the 20th generation (Series I). Dilutions up to 10^{-8} were made and six eggs were inoculated on to the chorio-allantoic membrane from each dilution. Incubation was continued for eight days after which the membranes were removed and examined. A characteristic thickening and oedema was present in eggs inoculated with all dilutions. Tubes of serum broth were also inoculated from each dilution and growths obtained up to 10^{-7}.

A similar titration with yolk removed from a 30th passage egg (Series II) was carried out and organisms demonstrated in dilutions up to 10^{-5}.

Further titrations have been carried out from time to time and deaths have regularly occurred in eggs inoculated with dilutions of 10^{-8} (the highest dilution so far used).

From these preliminary investigations it is evident, therefore, that organisms are present to a high titre in chorio-allantoic membrane, embryo, allantoic fluid and to a lesser extent, in yolk, using either eggs or serum broth as an indicator.

Temperature of Incubation.—The effect of varying the temperature of incubation on the rate of growth of C.B.P.P. organisms in eggs was next examined. Deaths of embryos occurred rather sooner and more consistently at 37° C. than at 35° C., and there was marked superiority with both these temperatures over 33·5° C.

Time of Harvesting.—Should the results of the present work be sufficiently favourable to encourage the use of egg-adapted strains of C.B.P.P. organisms as immunising agents, the optimum time of harvesting dead embryos would assume considerable importance. With this in mind, a rough trial was carried out by harvesting generation 41 (Series III) embryos dying on the second, third, fourth and fifth days after inoculation. The embryos were sub-inoculated in the usual manner. There appeared to be no significant difference between the results from embryos of different ages, deaths following in each case within the usual period.

Inoculations in Cattle

The characters of the local reactions of cattle to avianised C.B.P.P. organisms, whether fresh, dried or after one or two sub-cultures in serum broth were similar to those which follow the use of "culture vaccine" of varying degrees of virulence. In general, reactions began 7 to 10 days after inoculation, small swellings resolved in a further 7 to 10 days but large swellings persisted for longer periods. Where death occurred the course and character of the lesions were generally similar to those caused by C.B.P.P. "lymph." The first animal inoculations with egg-passaged strain T1 were made at the ninth generation (Series I. Table VII). One animal received allantoic fluid one undiluted chorio-allantoic membrane and six a second generation culture in B.S.B. of organisms from the chorio-allantoic membrane. No local reactions occurred. No. 1583 was not challenged but of the six challenged with "lymph" of proven virulence, none reacted.

At the 15th generation (Series I) three animals were given a first generation culture (B.S.B.) of organisms from the chorio-allantoic membrane. One (1137) reacted severely but recovered; neither of the others developed swellings; when challenged there were no reactions.

At the 28th passage (Series II), of four animals given embryo, one developed a large local swelling which

resolved. The immunity of this group was not proved since the challenge "lymph" failed to cause reactions in the controls.

At the 30th passage (Series III) local reactions occurred in three out of three animals given 1·0 c.c. of whole egg; all were resistant to challenge.

At the 31st passage (Series II) using whole egg, less yolk, one animal died as a result of a dose of 10 c.c. and one given 1·0 c.c. had no local reaction to vaccination and was resistant to challenge.

At the 32nd passage (Series II) 13 cattle received whole egg which had been lyophilised, stored for eight days at −20° C. and reconstituted with normal saline to its original volume. Eight developed small swellings. Twelve of the 13 cattle were challenged, one showed no reaction but died 13 days later from other causes, the remainder either did not react or the swellings were small and soon subsided.

Using egg material from Series III further groups of cattle were inoculated at the 33rd, 34th and 38th generations. In the first group (generation 33) none had local reactions but of four animals challenged two died, one reacted and recovered and one did not react.

In the second group (generation 34) one out of six developed a local swelling which was unusual in that it enlarged and resolved very slowly. When challenged there were no reactions.

In the third group (generation 38) six cattle out of 10 developed small local swellings following vaccination. The animal used as a control did not react to challenge nor did the vaccinated cattle; the immunity test was, therefore, inconclusive.

Titrations in Cattle.—At the 30th passage (Series III) a titration was carried out using whole egg (Table II). Dilutions were carried to 10^{-5}. Moderate local reactions occurred in the group receiving 1·0 c.c. of undiluted egg; no local reactions occurred in the remainder. The cattle in the undiluted and 10^{-1} groups were challenged after 28 or 45 days and no reactions occurred. The animals in the other groups were challenged at 28, 38 or 59 days. At 28 days all but the 10^{-4} animals died. At 38 days the 10^{-5} and 10^{-3} animals died while those given dilutions of 10^{-2} and 10^{-4} did not react. At 59 days those given dilutions of 10^{-5} and 10^{-4} did not react: the 10^{-2} animal reacted and recovered and the 10^{-3} animal died.

At the 31st passage (Series II) cattle were given 10·0 c.c., 5·0 c.c. and 1·0 c.c. of undiluted whole egg, less yolk, and 1·0 c.c. of 10^{-1} to 10^{-5} dilutions (Table III). Deaths resulted in the animal receiving 10·0 c.c. and in one of the three receiving a dilution of 10^{-1}. A severe local reaction occurred in one of three animals receiving the 10^{-2} dilution. Reactions among the remainder were small or did not occur. When challenged after 34 days, the animals in the undiluted, 10^{-1} and 10^{-2} groups survived after showing moderate local reactions. One animal was challenged in each of the three groups receiving the higher dilutions and all died.

TABLE I

ANIMAL INOCULATIONS WITH STRAIN T1, EGG-ADAPTED ORGANISMS

Animal	Egg generation	Inoculum	Dose, c.c.	Reactions		Challenge		
				Local. Inches	Complement fixation. Titre, days P.I.	Interval. Days	Local reactions. Inches	Reaction of controls
Egg Series I								
1595	9	Allantoic fluid	2·0	Nil	−ve 11, 18	33	1 × 1	Nil (1)
1583	9	C.A.M.	"	"	2/10 11 −ve 18		Not done	
1837	9	2nd gen. B.S.B., ex C.A.M.	1·0	"	2/10 11, 18	59	Nil	1 died 1 K.I.E.
1960	9	"	2·0	"	−ve 14	56	"	
1894	9	"	"	"	2/20 16	56	"	
1907	9	"	"	"	2/20 14	56	"	
1935	9	"	"	"	−ve 14	56	"	
1835	9	"	"	"	2/40 14	56	"	
1137	15	1st gen. B.S.B., ex C.A.M.	1·0	18 × 6	2/10 3, 10	56	"	1 died 1 K.I.E.
1929	15	"	"	Nil	−ve 10	56	"	
1947	15	"	"	"	−ve 10	56	"	
Egg Series II								
2198	28	Embryo	2·0	"	−ve 11 1/40 18 1/80 25	32	"	Nil (1)
1927	28	"	1·0	1 × 1	2/40 32 −ve 18 3/20 25	32	"	
2228	28	"	"	8 × 3	2/40 25	42	"	
2221	28	"	"	Nil	−ve 11, 18 2/20 25	32	"	

TABLE I—*continued*

Animal	Egg generation	Inoculum	Dose c.c.	Reactions: Local. Inches	Reactions: Complement fixation. Titre, days P.I.	Challenge: Interval. Days	Challenge: Total reactions. Inches	Challenge: Reaction of controls
EGG SERIES III								
2595	30	Whole egg	1·0	4 × 8	2/20 14 1/40 4/40 21	28	„	3 K.I.E. 1 reacted and recovered
2566	30	„	„	3 × 1	2/20 14 1/40 4/40 21	28	„	
2585	30	„	„	5 × 1	3/20 14 1/40 4/40 31	45	„	No control
EGG SERIES II								
2111	31	Whole egg, less yolk	10·0	Died				—
2105	31	„	1·0	Nil	2/10 12	34	2 × 1	3 K.I.E., 1 reacted and recovered
1	32	Whole egg (dried)	1·0	„	−ve 10, 14, 21	47	Nil	3 K.I.E., 2 reacted and recovered
2	32	„	„	„	−ve 10, 14, 21	47	„	
3	32	„	1·0	1 × 1	−ve 10, 14, 21		Not done	
4	32	„	„	4 × 4	3/10 10	47	1 × 1	
5	32	„	„	2 × 2	2/10 1/20 10 −ve 14, 21	47	1 × 1	
6	32	„	„	1 × 1	2/10 10 −ve 14, 21	47	Died other causes	
7	32	„	„	Nil	−ve 10, 14, 21	47	2 × 3	3 K.I.E., 2 reacted and recovered
8	32	„	„	½ × ½	−ve 10, 14, 21	47	Nil	
9	32	„	„	1 × 1	1/10 10 2/20 14 −ve 21	47	½ × ½	
10	32	„	„	½ × ½	−ve 10, 14, 21	47	Nil	
11	32	„	„	Nil	2/10 1/20 10, 14 −ve 21	47	„	
12	32	„	„	2 × 2	A/C 10 1/10 14	47	2 × 2	
13	32	„	„	Nil	−ve 10, 14, 21	47	Nil	
EGG SERIES III								
2760	33	Whole egg, less yolk (dried)	„	„	−ve 14	39	Died	2 K.I.E.
2764	33	„	„	„	−ve 14	39	„	
2783	33	„	„	„	−ve 14	39	Nil	
2740	33	„	„	„	−ve 14	39	4 × 4	
2615	33	„	„	„	−ve 14	28		No control
2769	33	„	„	„	−ve 14	28		
2787	33	„	„	„	−ve 14	28		
2674	33	„	„	„	−ve 14	28		
2725	34	Embryo and fluids (dried)	„	„	−ve 14	28	Nil	2 K.I.E., 1 reacted and recovered
2667	34	„	„	„	2/20 14 −ve 21	28	„	
2774	34	„	„	„	−ve 14	28	„	
2728	34	„	„	4 × 4*	3/40 14 3/20 24	45	„	No control
2754	34	„	„	Nil	−ve 14	31	„	1 K.I.E., 1 reacted and recovered
2705	34	„	„	„	−ve 14	31	„	
3799	38	Whole egg (dried)	„	½ × ½	1/10 14	28	„	Nil (1)
2792	38	„	„	1 × 1	1/20 14	28	„	
2789	38	„	„	Nil	−ve 14	28	„	
2692	38	„	„	1 × 1	−ve 14	28	„	
2680	38	„	„	„	−ve 14	28	„	
2772	38	„	„	„	−ve 14	28	„	
2862	38	„	„	Nil	−ve 14	28	„	
2825	38	„	„	Died other causes				
2843	38	„	„	1 × 3	−ve 14	28	„	
2742	38	„	„	Nil	3/10 14	28	„	

Note. * = Reaction delayed until 28th day.
A/C = Anti-complementary.
B.S.B. = Bovine serum broth.
K.I.E. = Killed *in extremis*.
P.I. = Post-inoculation.

TABLE II—TITRATION IN CATTLE OF STRAIN Tl, WHOLE EGG, 30TH PASSAGE. SERIES III. DOSE 1·0 C.C.

Animal	Dilution	Reactions: Local. Inches	Reactions: Complement fixation. Titre, days P.I.	Reactions: Interval. Days	Challenge: Local reaction Inches	Challenge: Reactions of controls
2595	Undiluted	4 × 8	2/20, 1/40 } 14 4/40 21	28	Nil.	3 K.I.E. 1 reacted and recovered
2566	"	3 × 1	2/20, 1/40 } 14 4/40 21	28	"	
2585	"	5 × 1	3/20, 1/40 } 14 4/40 21	45	"	No control.
2574	10^{-1}	Nil	2/10, 1/20 } 14 −ve 21	28	"	3 K.I.E. 1 reacted and recovered.
2597	"	"	−ve 14 3/20 21	28	"	
2564	"	"	3/10, 1/20 } 14 −ve 21	45	"	No control.
2571	10^{-2}	"	−ve 14, 21	28	Died	—
2575	"	"	−ve 14, 21	38	Nil	2 K.I.E.
2570	"	"	−ve 14, 21	59	8 × 8	2 K.I.E.
2567	10^{-3}	"	−ve 14, 21	28	Died	—
2552	"	"	−ve 14, 21	38	"	—
2549	"	"	−ve 14, 21	59	"*	—
2556	10^{-4}	"	−ve 14, 21	28	6 × 4 Recovered	3 K.I.E., 1 reacted and recovered.
2594	"	"	−ve 14, 21	38	Nil	2 K.I.E.
2590	"	"	−ve 14, 21	59	"	2 K.I.E.
2589	10^{-5}	"	−ve 14, 21	28	Died	3 K.I.E., 1 reacted and recovered.
2557	"	"	−ve 14, 21	38	"	2 K.I.E.
2553	"	"	−ve 14, 21	59	Nil	2 K.I.E.

* = Death after delayed action.

TABLE III—TITRATION IN CATTLE OF STRAIN Tl, WHOLE EGG LESS YOLK, 31ST PASSAGE, SERIES II

Animal	Dose, c.c.	Dilution	Reactions: Local. Inches	Reactions: Fixation complement. Titre, days P.I.	Reactions: Interval. Days	Challenge: Local reaction. Inches	Challenge: Reaction of controls
2111	10·0	Undiluted	Died		—	—	—
2118	5·0	"	Died other causes		—	—	—
2105	1·0	"	Nil	2/10 12 −ve 19, 26	34	2 × 1	3 K.I.E., 2 reacted and recovered
2113	"	10^{-1}	"	−ve 12, 19	34	1 × 1	
2109	"	"	Died	1/10 12 −ve 19 3/80 26	—	—	—
1521	"	"	½ × ½	1/10 12, 19, 26 −ve 26	34	3 × 4	3 K.I.E., 2 reacted and recovered.
2096	"	10^{-2}	10 × 12	−ve 12, 19 2/40 26		Not challenged	
1530	"	"	½ × ½	−ve 12 1/10 19 −ve 26	34	6 × 6	3 K.I.E., 1 reacted and recovered.
1532	"	"	Nil	−ve 12 1/10 19 −ve 26	34	2 × 2	
2101	"	10^{-3}	"	−ve 11, 18, 25	34	Died*	
2110	"	"	"	−ve 11, 18, 25		Not challenged	
1535	"	"	"	−ve 12 2/10 19 −ve 26		"	
1520	"	10^{-4}	"	−ve 11, 18, 25	34	Died†	3 K.I.E., 2 reacted and recovered.
1527	"	"	"	−ve 11, 18, 25		Not challenged	
2120	"	10^{-5}	"	−ve 11, 18, 25	34	Died	3 K.I.E., 2, reacted and recovered.
2102	"	"	"	−ve 11, 18, 25		Not challenged	
2094	"	"	"	−ve 11, 18, 25		"	

* Developed large static swelling. Destroyed on humanitarian grounds 30 days after challenge. Might have recovered.
† Developed large static swelling. Destroyed 24 days after challenge.

Discussion

Growth in eggs was readily obtained with the strain used and macroscopic changes in the chorio-allantoic membrane were observed in early generations. A general invasion of embryonic tissues occurred and organisms to a high titre were recovered from membranes, embryos and fluids. Although deaths of embryos occurred following inoculation by the chorio-allantoic membrane route, more constant results were obtained by the yolk sac route, deaths occurring regularly on the third and fourth days.

It is of interest to note that Series III was commenced with chorio-allantoic membrane that had been dried and stored at −20° C. for nearly four months. The organisms appeared still to be fully virulent and growth in eggs was readily obtained. This is an important point since, as will be discussed later, the superior value of this strain for vaccine purposes will largely depend on its ability to withstand storage.

From the few titration experiments carried out it is evident that organisms were present in chorio-allantoic membrane, embryo, allantoic fluid and, to a lesser extent, in yolk, to a high titre for eggs. Rough estimations were made of the optimum temperature of incubation and the optimum day for harvesting, from which it appeared that the higher temperatures favoured maximum growth, whilst organisms were recovered from embryos dying from the second day onwards.

The results of animal inoculations are, in some respects, difficult to evaluate. For example, satisfactory reactions did not always occur in control animals, either because they were resistant or because the challenge material was not sufficiently virulent. Thus firm conclusions cannot be drawn following the challenge of 14 animals inoculated at the 9th, 28th and 38th egg generations. Again, in a number of cases, challenge inoculations were followed by small local reactions and recovery, and it may be argued that these animals had not been fully immunised. In such cases, exposure to natural infection in the field might change a state of partial immunity into one of chronic infection. The assessment of the relationship between the subcutaneous method of challenge with "lymph" and natural infection is a problem in itself. It is generally accepted, however, that this method of challenge is more severe than natural exposure and that the absence of any reaction to inoculation with "lymph" indicates a satisfactory immunity against C.B.P.P. In the present experiments this has been achieved in a large proportion of animals, and, in certain batches, in all animals.

With one exception (generation 33), the avianised strain of T1 conferred an immunity at least comparable with that following the use of culture vaccine. This immunity was engendered with a single subcutaneous dose of 1·0 c.c. given behind the shoulder. When it is added that out of 32 animals successfully immunised only two (1137 and 2228) showed local reactions to the vaccine likely to cause concern to the stockowner, it will be appreciated that there are firm grounds for hope that further experimentation may result in a vaccine superior to any yet produced for the control of pleuropneumonia in cattle. Some comparisons may be made between our results and those following the use of culture vaccines (Table IV).

Rougentzoff (1913) used a virulent culture by tail inoculation. Purchase (1939) used a safe generation of culture vaccine, usually by shoulder inoculation, and varied the number of doses. In all three cases the method of challenge was substantially the same. The figures for avianised strain T1 do not include animals used at generation 33, which will be discussed later, nor those animals for which there was no adequate control of challenge material. When it is remembered that with "lymph" and culture vaccines inoculated into the tail, mortality and loss of tails may be high, it can be said that the avianised strain has so far proved at least as effective and very much safer.

Table IV

	Challenged	Died	Reacted and recovered	No reaction
Rougentzoff	114	7 (6%)	48 (42%)	59 (52%)
Purchase	143	34 (24%)	63 (44%)	46 (32%)
Avianised strain T1	32	0	8 (25%)	24 (75%)

Certain features of interest may be discussed briefly. The strain was found to be sufficiently attenuated at the ninth passage to cause solid immunity in cattle without local reaction. So far, no further lessening of virulence has occurred. In fact, material from the 32nd, 34th and 38th passages caused local reactions.

The titration in cattle of whole egg, generation 30 (Series III), showed that a dilution of 10^{-1} conferred satisfactory immunity. Furthermore, local reactions following vaccination of this amount were entirely absent. Some protection was afforded at higher dilutions, particularly when the interval between inoculation and challenge was lengthened.

The titration with 31st passage whole egg less yolk produced unexpected results and it will be noted that the material used was from Series II, in which egg to egg passage had been interrupted by sub-culturing in serum broth at the 25th, 28th and 31st passages. We believe that modification by egg passage may perhaps be reversed by returning the organism to serum broth. One further passage in eggs, however, appeared to restore the strain to approximately its previous virulence. Thus, when generation 32 was tested in cattle, local reactions were absent or small and a satisfactory immunity resulted. It seems, therefore, that at least during the early stages of modification, the organisms are in a state of delicate balance and may easily be affected one way or the other according to the medium in which they are grown.

Unexpected variations in virulence have always constituted an unpredictable hazard with culture vaccine and we believe that an uninterrupted modification by egg passage may overcome this undesirable feature. Furthermore, should avianised strains be used for vaccine production, by drying and storing successive batches, each one could be tested for safety and efficacy before issue. Large doses are inclined to cause excessive local reactions and very small doses usually fail to immunise. By titrating each batch in cattle, it would be possible to select a dilution able to confer a solid immunity with little or no reaction.

The severe local reaction observed in bovine 1137, given egg generation 15 material, calls for brief comment. This animal was bled three days after inoculation and showed a low C.F. titre which remained at the same level for seven days. It is possible, therefore, that it was suffering from a chronic infection of C.B.P.P. at the time and that this was responsible for the violent reaction to the vaccine. Experiments designed to estimate the effect of vaccinating "carrier" animals will form the subject of a subsequent article.

An unexpected result followed the use of egg generation 33 (Series III). There were no reactions amongst the eight cattle used, but when four of them were challenged, two died, one reacted and recovered and one was resistant. The reason for this is not yet understood.

There was no apparent connection between the severity of reactions to different egg generations of the strain used and the resulting immunity; in fact, generation 31 (Series II), which was more virulent than generation 30 (Series III), appeared to afford a rather less solid immunity.

A study of the C.F. titres of vaccinated animals does not appear to yield information of significant value. There seemed to be no correlation between titres and immunity. In general, they were lower than those which follow culture vaccines, whereas the immunity was of a much higher order. Antibodies were always demonstrable in animals which developed local swellings considered to be specifically due to the C.B.P.P. organism. On the other hand, a proportion of animals showed antibodies in the absence of local reactions and others showed neither.

Summary

The work reported in this article indicates that strains of the C.B.P.P. organism can be grown in embryonated eggs without difficulty. With the strain used (T1), cattle were immunised successfully after nine egg passages by subcutaneous inoculations which were rarely followed by undesirable local reactions. Using a dose of 1·0 c.c. of egg material, 20 to 40 per cent. of cattle developed local reactions, but further work, which will be published later, has shown that this dose can be reduced to a point at which local reactions are almost entirely absent whilst immunising value is not impaired. Higher dilutions may result in a slowly developing and generally low grade immunity. Within these extremes a high grade and uniform immunity can be established within 28 days with few local reactions. Provided the keeping qualities of dried vaccine prove to be satisfactory, it should be possible to select, by animal titration, a dose adequate to meet these requirements whilst allowing a safe margin of immunising doses to cover any variation in the susceptibility of cattle.

Conclusions

1. A field strain (T1) of C.B.P.P. organisms has been grown in embryonated eggs.

2. By the ninth egg passage cattle were satisfactorily immunised with a single subcutaneous dose of organisms cultured from infected chorio-allantoic membranes against challenge with "lymph" of proven virulence. Further successful results were obtained with whole egg and embryo.

3. No further lessening of virulence appeared to occur between the 9th and 34th egg passages.

4. Local reactions were generally absent or moderate in size.

5. Apart from occasional variations in its virulence, the reasons for which are under active examination, the avianised strain T1 shows promise of being a safer, more stable and more effective immunising agent than those at present in use for the control of contagious bovine pleuropneumonia.

Acknowledgments.—It is a pleasure to acknowledge the valuable technical assistance received from Mr. A. J. Wiley, laboratory assistant, and Miss E. M. Afford, microscopist. This paper is published with the permission of the Director of Veterinary Services, Kenya.

HERD FUNCTIONAL INFERTILITY, WITH REFERENCE TO NUTRITION AND MINERAL INTAKE

BY

J. G. WILSON, M.R.C.V.S.,

Veterinary Investigation Laboratory, Starcross, Devon

When a study of herd infertility was made in the south-west of England during the years 1944-47 it was found that the majority of the outbreaks were associated with *Brucella abortus* and *Trichomonas foetus*. Clinical examinations of large numbers of cows revealed abnormal conditions of the genital tract, such as catarrhal endometritis, cervicitis and vaginitis. Since then a new type of herd infertility was recognised and seemed to become more and more prevalent. In this type there was no evidence of infection of the uterus, cervix and vagina but in the majority of cases there was evidence of ovarian dysfunction (Wilson, 1949).

In the herds most severely affected there was a high proportion of anoestrum, especially in the heifers, although in some herds adult cows were anoestrous even in the summer months.

In less severe cases the ovaries were found to be very small, as in anoestrum, although the cows were having oestrous cycles. In herds which appeared to recover it was common to find one ovary very small and inactive and the other functioning normally. In other cases a follicle instead of a corpus luteum was often found at mid-cycle, indicating ovulatory failure, and in a few cases an atypical corpus luteum was found which was fluctuating and could not be expressed cleanly—the "cystic" corpus luteum or possibly a luteinised follicle which had failed to ovulate.

In some herds suspected of functional infertility no definite ovarian dysfunctions could be detected. Further experience has suggested that some of these cases were associated with *Vibrio foetus* infection. Jersey, Guernsey and Ayrshire herds appeared to be much more commonly affected by this type of infertility than the native Devon and South Devon breeds which predominate in the south-west, suggesting a breed predisposition.

The trouble appeared to be commoner in recently established herds than long established herds of the same breed, suggesting an acclimatisation factor. As the trouble appeared to be functional rather than infective in nature, some other environmental cause was sought and, as in a number of the affected herds first investigated, ploughing up, liming and the establishment of leys had been undertaken, the possibility of the high soil calcium status rendering manganese unavailable was first explored but did not yield a solution.

The services of the N.A.A.S. were then sought, and in a number of cases in which the rations were analysed a low protein combined with a low phosphate content of the herbage and bulk feeding-stuffs was found. It appeared at first that the two were always

References

Campbell, A. D., & Turner, A. W. (1936.) Commonwealth of Australia, C.S.I.R. Bulletin No. 97 ; pp. 11-52.
Piercy, S. E. (1952.) *J. comp. Path.* **62.** 152.
Purchase, H. S. (1939.) *Vet. Rec.* **51.** 2 and 3, 31-47 and 67-75.
Rougentzoff, D. (1913.) *C.R. Soc. Biol.* **75,** pt. I. 271-273.
Walker, J. (1919-22.) An. Reps. Dept. Agric., Kenya, 1919-20, 1921 & 1922.

associated with this condition and accounted for the anoestrous states (Linton, 1951). Later in the investigation, however, it was found that cows in herds on a high protein intake presented the same picture of anoestrum and small ovaries. At about this time Hignett (1949, 1950) published papers on the subject putting forward his Ca:P ratio hypothesis. The latter appeared to offer a possible solution and after consultation with Mr. Hignett and with Mr. Holt (of Dartington Hall Cattle Breeding Centre) it was decided to carry out a controlled mineral feeding experiment on some of the "problem" herds associated with the Centre. (By using artificially inseminated herds it was hoped to rule out the sub-fertile bull, and also the known venereal diseases.)

In a survey of the herds covered by this Centre and its sub-centres at Tavistock and Liskeard, it was found that

Results of Controlled Mineral Supplement Experiment

Involving 16 "Problem" A.I. herds—Fed from November 1st, 1950, to June 30th, 1951
Results of 11 herds out of 16 starters which completed the experiment

Conception rates in services per conception

Number of herd	Breed	1950 total	1951 total	Group A controls. (Number of cows)	Group B, phosphate. (Number of cows)	Group C, trace elements. (Number of cows)	Remarks
3	Mixed, Ayr and G.	2·9	1·8	(2) 2	(2) 2·5	(7) 1·6	—
4	Ayr	2·5	2·7	(4) 1·5	(3) 3	(3) 3·5	6 lb. hay and 3 lb. straw not included. Very high protein intake. Two cows, recorded as "A" group, had minerals after service (1B, 1C) and held to first service. One cow in Group B had endometritis and did not conceive.
7	Ayr	2·3	1·9	(5) 2·2	(1) 1	(2) 1·5	—
8	Ayr	3·1	1·7	(4) 1·5	(5) 2	(5) 1·5	Infertility on silage in 1950. No trouble on silage in 1951.
9	Ayr	3·1	1·9	(4) 1·7	(3) 1·4	(4) 2·3	Neighbouring farm to "8" with similar history
10	Ayr	3·4	2·8	(4) 2·2	(3) 5	(3) 2	Low state of nutrition. Poor milk yield. Five cows had long periods of anoestrus.
13	Ayr	3·6	1·9	(4) 1·75	(4) 2·4	(4) 1·5	Less kale fed than in 1950.
15	G.	3	1·5	(5) 1	(7) 1·6	(3) 1	Put his problem cows in Group "B."
6	Ayr	2·9	1·8	(4) 3·25	(4) 1	(4) 1	Apparently clear response in Groups B and C. Heavy kale feeding.
14	Ayr	3·25	1·9	(4) 2·25	(3) 2	(3) 1·25	—
16	Ayr	2	1·4	(4) 2·25	(5) 1	(4) 1	Some of the phosphate in the ration in form of fish meal.

Summary of Results in above 11 herds

Group	"A" (controls)	"B" (phosphate)	"C" (trace elements)
CR (1) Services per conception	1·95	1·83	1·54
(2) % holding to first insemination	37·2%	50%	61·9%

Of the above 11 herds, three herds—Numbers 6, 14 and 16—showed an apparently clear response to mineral therapy. Of the remaining eight, only in two (Numbers 4 and 10) was there any indication of infertility persisting. In both of these nutrition was faulty. Very high protein in No. 4 and very poor nutrition in No. 10.

Summary of Results in the three herds with response to minerals

Group	"A"	"B"	"C"
CR (1) Services per conception	2·6	1·1	1·1
(2) % holding to first insemination	16·7%	75 %	91%

It is interesting that out of six cows which had had 37 unsuccessful services between them prior to joining the experiment, three held to first service after feeding with phosphate and the other three held to first service after trace element feeding. The worst case had been barren for over a year and had had 15 previous services.

out of some 4,000 herds there were at that time about 30, *i.e.*, less than 1 per cent., with herd infertility as defined by Hignett (1950), *viz.*, " herd in which a third or more of the herd needs three or more services for conception." Of these, the herds with a recent history of contagious abortion, the herds which were too small to give a reasonably significant number of cows, and herd owners who would not co-operate were discarded. Sixteen herds involving a possible 240 cows were included in the experiment; the breed distribution was 12 Ayrshire herds, two Guernsey, one South Devon and one mixed Ayrshire and Guernsey.

The experiment was planned to run for about six months from the beginning of November, 1950, to about the end of April, 1951, and analysis of the winter rations was arranged.

Each herd was divided by taking alternate cows as they became ready for breeding and putting them into one of the following three groups:—

Group "A"—Controls.

Group " B "—(Phosphate). Each animal was given 3 oz. of acid sodium phosphate daily in the food.

Group "C"—(Trace elements). Each animal was given a mixture containing 15 gm. manganese sulphate, 1 gramme copper sulphate and 240 mg. potassium iodide per day in the food.

Each cow was to be on the experiment for nine weeks, being fed for three weeks before the service to count. This allowed for up to three services during the experimental period.

Most of the cows came straight on to the experiment after calving—feeding commencing at the first observed oestrus *post-partum*, and service following at the next oestrus.

A few "difficult" cows which had been served before were allowed to miss one service; feeding then commenced and they were served at the following oestrus.

Results

Of the 16 herds, two owners gave up at an early stage and three more did not carry out the conditions of the experiment.

The results of the remaining 11 herds are given on the table on page 622.

Conception rates shown were obtained by pregnancy diagnosis.

Discussion

It is interesting to note that in the herds that apparently responded to mineral therapy a response was elicited both by *phosphate* and by *trace element* supplements with slightly better results with the trace element supplement, although in most cases a wide $CaO:P_2O_5$ ratio and/or phosphate deficiency was indicated by the analyses of the rations.

It was thought at first that one of the trace elements might be necessary for the optimum absorption and utilisation of phosphorus. In view of the findings in an experiment by Shirley *et al.* (1950), who showed that high copper or molybdenum intake affected the absorption, deposition and excretion pathway of radio-active phosphorus, it was thought that the trace element might possibly be copper, enabling the cow to make better use of the phosphorus in the diet. It was also considered that the supplying of extra phosphorus, by increasing the absolute amount, might have rectified the deficiency and cured the infertility.

Later, consideration was given to another hypothesis which appeared to fit the results more closely—*viz.*, that a deficient trace element caused the infertility, that this was especially aggravated by a high Ca status, and that the condition could be rectified either by supplying excess of the deficient trace element, or excess phosphate, to counteract the high Ca status.

Herd No. 6, which had the highest Ca intake and the clearest response to both phosphate and trace elements is being divided into three groups this winter and each group fed a single one of the three trace elements (I, Cu and Mn) in the original Group C mixture.

There is some difference of opinion as to the P_2O_5 requirements of dairy cattle. Linton (1951) suggests 40 g. of CaO and 22 g. of P_2O_5 per 1,000 lb. live-weight for maintenance and 17·4 g. CaO and 21·8 g. P_2O_5 per gallon of milk for production with a $CaO:P_2O_5$ ratio of 1·3:1. Hignett and Hignett (1951) consider that, for high fertility, there should be a P_2O_5 intake of 50 gm. for maintenance and 19 g. per gallon for production with a CaO intake exceeding that of P_2O_5. It is of considerable interest that although most of the text-books on nutrition indicate that the diet of the dairy cow is likely to be richer in phosphorus than in calcium, our analyses of rations in the south-west have indicated that the imbalance is much more frequently in favour of calcium, and that a $CaO:P_2O_5$ ratio of 2:1 is quite a common finding, especially in herds with functional infertility.

Acknowledgments.—The author is deeply indebted to Sir Thomas Dalling for his keen interest, help and guidance; to Mr. S. L. Hignett, of The Wellcome Veterinary Research Station, for his advice and help; to Mr. A. F. Holt, of Dartington Hall Cattle Breeding Centre, for his advice and collaboration and for the help of his staff in distributing the mineral supplements; to Dr. Stopher and Mr. E. L. Smith for co-operation in carrying out the experiment and for undertaking all the analyses of the foodstuffs and calculating the mineral intakes.

References

Hignett, S. L. (1949.) Paper to XIVth International Vet. Congress, London.
———. (1950.) Paper to 68th Annual N.V.M.A. Congress, Cardiff.
———, & Hignett, P. G. (1951.) *Vet. Rec.* **63.** 603.
Linton's Veterinary Dietetics. Third edition; Abrams, 1951. Pages 68 and 305.
Shirley, R. L. (1950.) *J. Anim. Sci.* **9.** 552.
Wilson, J. G. (1949.) *Brit. Vet. J.* **105.** 5.

Weekly Wisdom

Until our agricultural policy is treated at least as seriously as our foreign policy, we shall not be facing realistically the deadly situation in which a nation, unable to rely on safe food imports in sufficient quantities, and with only one-third of an acre of good land per head of population, now finds itself.—The Observer.

* * * * *

A puff adder which was seeking shelter from the rain in a Bindura bar met an unexpected end at the hand of the local darts champion. Mr. Colin Browne. The snake, which had crawled into the hotel unseen, was about to strike a resident, Mr. H. Shumbling, when Mr. Browne, in the middle of a match, saw it and hurled a dart through its head.—*The Times.*

ANATOMICAL COMMUNICATION

A NOTE ON A CASE OF FOUR HEARTS IN A HEN

BY

M. S. E. MOSTAFA, M.V.Sc.,

LECTURER IN DEPARTMENT OF PATHOLOGY, FACULTY OF VETERINARY MEDICINE, FOUAD 1ST UNIVERSITY, CAIRO, EGYPT

As far as the writer is aware, this case has not been reported previously and it seems worth while to place it on record with an illustration and a short description.

The fowl in which the four hearts were present was a Fayoumi hen. It was bought by a British lady for cooking. On opening the abdomen and finding the four hearts, she kindly sent me the specimen for examination.

The specimen, consisting of liver and four hearts, was 6·25 cm. long by 5·5 cm. wide. Naked-eye examination revealed a fatty liver, on the visceral surface of which four hearts were seen; two completely separated from one another and two united by a pedicle of connective tissue on the other side. They were normal in size and shape as shown in the accompanying photographs (Figs. 1 and 2).

Each of these hearts consisted of four chambers: a right and left atrium and a right and left ventricle. The walls of the atrial portion were thin while those of the ventricles were much thicker. The atria were separated from the ventricles by a muscular plate and membranous flaps corresponding to the tricuspid and mitral valves of mammals on the right and left sides. The opening from the right atrium into the right ventricle was crescentic in outline, while that of the opening from the left atrium into the left ventricle was circular. A transverse section was made through the ventricular mass. The wall of the left ventricle was very much thicker than that of the right and the septum between the two cavities bulged towards the right, resulting in the circular left cavity and the crescentic right one.

FIG. 1.—Photograph showing parietal aspect of the liver and the four hearts. Magnification approximately one and a half times normal.

FIG. 2.—Photograph showing visceral aspect of the specimen. Observe the two united hearts on the left lower side of the photograph. Note the shape and size of the four hearts. Magnification approximately one and a half times normal.

Microscopically, the cardiac muscle was normal and showed the striped appearance of the muscle fibres (Fig. 3). The liver showed a little fatty change but was otherwise normal.

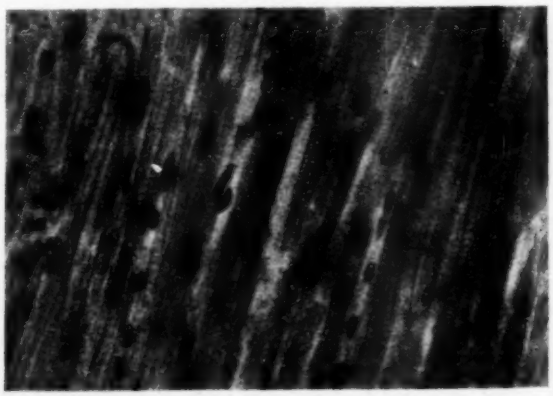


FIG. 3.—Photomicrograph of fowl cardiac muscle, showing cross striation. Haematoxylin and eosin × 950.

It was difficult to determine how the blood circulated in these four hearts as a complete examination of the blood vessels of each heart was not possible. It seemed to me, however, that the blood circulated in each of the four hearts in succession. Unfortunately, no other details of the case are available.

I am indebted to my colleague M. Housni, Director of the Radiological Department, for the photographs.

Protection against **Feline Infectious Enteritis**

'Wellcome' brand Feline Infectious Enteritis Vaccine is again available. During the immediate pre-war years this vaccine had established a place for itself in affording protection against the acute and highly fatal disease of cats, infectious enteritis (Panleucopenia). Antigenic strains of this virus have again been isolated and the vaccine prepared from the tissues of susceptible animals infected with the virus.

While a single dose of 1 c.c. may give sufficient protection, it is always advisable that a second dose be given 10 to 14 days after the first. Cats of any age may be immunised but it is probably best to give the first injection when kittens are about six weeks old.

'Wellcome' Feline Infectious Enteritis Vaccine is issued in ampoules of 1 c.c. Further details and literature will be forwarded to members of the veterinary profession on request.

'WELLCOME' BRAND **FELINE INFECTIOUS ENTERITIS VACCINE**

PREPARED AT THE WELLCOME RESEARCH LABORATORIES

SUPPLIED BY
BURROUGHS WELLCOME & CO. (THE WELLCOME FOUNDATION LTD.)
183-193, EUSTON ROAD, LONDON, N.W.1. TEL. EUSTON 4477 and 18, MERRION SQUARE, DUBLIN, TEL. DUBLIN 64460

The Stilboestrol Injection with the lowest viscosity

Crookes Stilboestrol Injection, in ethyl oleate, is unique in its power of retaining a low, water-like viscosity. It can withstand more than 50° of frost without thickening and may, therefore, be administered with a fine needle even on the coldest mornings. Supplied in 15 or 30 c.c. rubber capped bottles, Crookes Stilboestrol Injection affords 10 mgs. of stilboestrol dipropionate per ml. Literature will be sent on request.

CROOKES
STILBOESTROL
(VETERINARY)

THE CROOKES LABORATORIES LIMITED · PARK ROYAL LONDON · N.W.10

SEE THE EVIL...

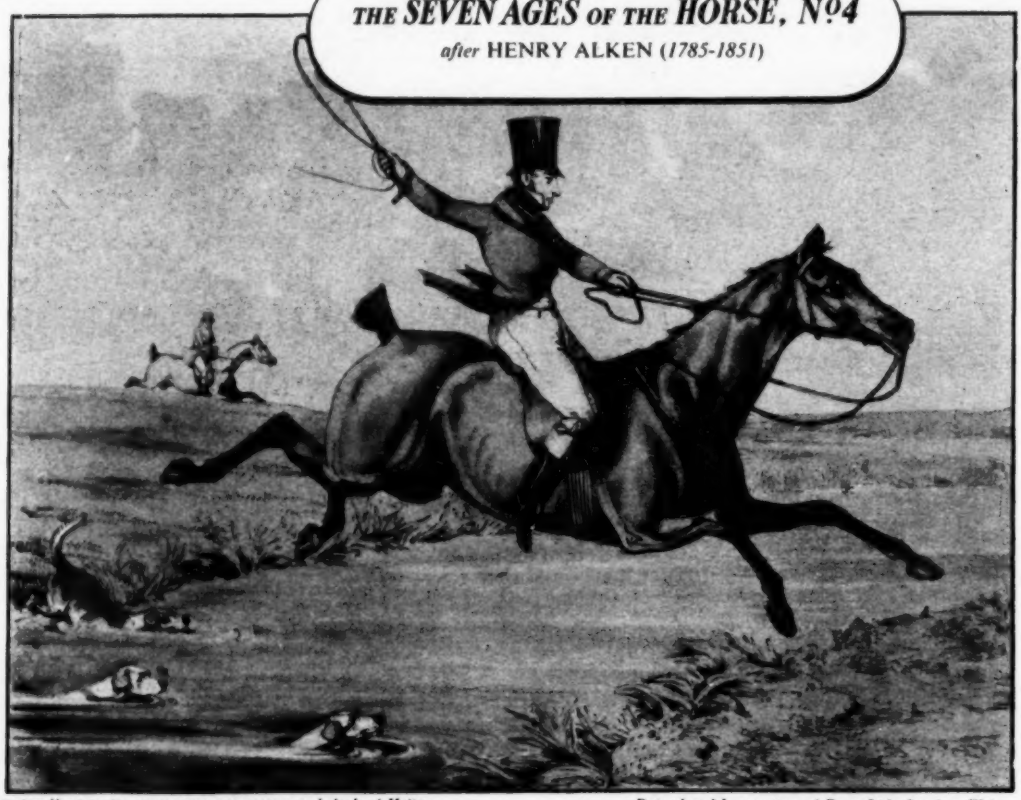
Diagnosis is the veterinary surgeon's problem; the cure, to some extent, is ours. The many orders we receive for veterinary specialities and dispensing supplies through our branches is a testimony to the high confidence with which veterinary surgeons specify Boots.

. . . cure the evil

Boots

THE SEVEN AGES OF THE HORSE, No4

after HENRY ALKEN (1785-1851)



Then the Hunter, his sinews stronger grown, and dock of Melton cut, jealous of being first sudden and quick in action, seeking the bubble reputation even at a seven yards brook.

Reproduced by courtesy of Fores Ltd., London, W.1.

In penicillin therapy **'DISTAQUAINE'** brand *preparations of procaine penicillin offer the following advantages:*

- ★ Aqueous, containing neither oil nor wax
- ★ Easy to prepare and administer
- ★ Least possible pain on injection
- ★ Effective blood levels up to 24 hours following administration
- ★ Dry syringe unnecessary
- ★ Equipment easily cleaned after use

'DISTAQUAINE' *SUSPENSION*
vials of 10 *ml.* (300,000 *units per ml.*).

'DISTAQUAINE' G
vials of 300,000, 900,000 *and* 3,000,000 *units.*

'DISTAQUAINE' *FORTIFIED*
vials of 400,000 *and* 1,200,000 *units.*

available from:

Allen & Hanburys Ltd.
British Drug Houses Ltd.
Burroughs Wellcome & Co.
Evans Medical Supplies Ltd.
Imperial Chemical (Pharmaceuticals) Ltd.
Pharmaceutical Specialities (May & Baker) Ltd.

'Distaquaine', a trade mark, is the property of the manufacturers

THE DISTILLERS COMPANY (BIOCHEMICALS) LIMITED

SPEKE LIVERPOOL

CLINICAL COMMUNICATION

EMBRYOTOMY OF SIAMESE TWINS IN A BUFFALO COW

G. S. CHINNIAH,
Veterinary Surgeon, Colombo, Ceylon

On December 25th, 1951, on examination of a valuable buffalo cow on a Colombo dairy farm which had shown the water bag but in which normal delivery did not occur, I found the left foreleg crossed over the back of the neck in the anterior position (an abnormal position in calving), the right foreleg bent back at the knee, and the head bent downwards towards the lower part of the womb. I introduced my arm more fully and found that the calf was lying in a transverse position with the right side upwards. During manipulation I also found the two inner hind legs, which I secured separately (these hind legs had subsequently to be amputated). I then manipulated the head into position and also secured the forelegs with running nooses. All this took nearly 45 minutes.

From a standing position the animal now lay down. After further examination and manipulation I decided to perform embryotomy in preference to a caesarean, and having obtained the owner's consent, proceeded with the operation.

Having applied Velufax Obstetric Lubricant (Burroughs Wellcome) on both hands I made sure that the running loops on the neck and forelegs were made tight. I now pushed the foetus as far as it would go into the womb. A dose of 3,000,000 units of penicillin was administered to the cow, which at an earlier stage had been given 2 oz. of chloral hydrate. On examination I found that the navel cord was abnormally large and as, on pulling, there were no signs of its yielding, I thought it might be wound over somewhere and left it alone. I now drew forward one of the right hind legs which had been secured at first. I then introduced the embryotome, pulled the hind leg through the barrel, and with my assistant holding the end of the rope which was secured, amputated the hind leg.

After re-examination I decided to cut the navel cord which was wound over one of the hind legs beyond reach, as I feared that the drag might cause an injury to the walls of the womb. This was done with the use of an embryotomy knife. I now pulled at the foetus, which moved forward. There was now less obstruction for closer examination and whilst manipulating I encountered the hind legs of the twin. At this stage there was definite evidence that it was a case of Siamese twin birth and I proceeded to deliver. The first calf died during the operation. Using more force, I gave another pull but my efforts were unsuccessful. I now pulled on the secured right hind leg which, on examination, I found was the right hind leg belonging to the other twin calf. Even at this stage I tried to deliver them individually but was unable to do so, as the twins were inseparably joined together from flank to flank in a position of trunk presentation.

After intense manipulation I now decided to amputate the already secured hind leg which was the next obstruction to delivery. The embryotome was again used and the hind leg was amputated and removed. The existing twist in the navel cord was relaxed, and the hind leg which I had previously encountered and spared was placed in position.

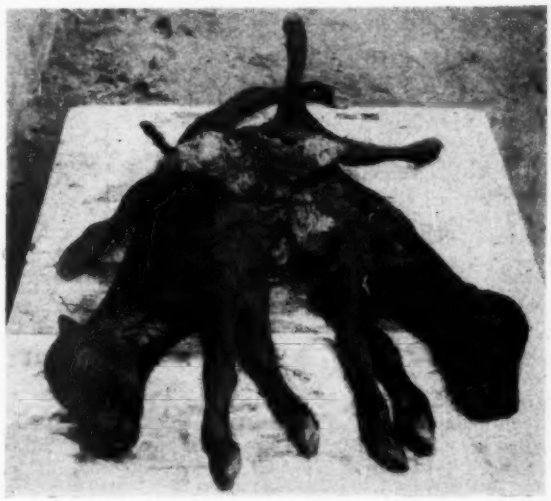
I was now able to feel one of the forelegs and head of the other twin, which were lying in anterior positions. The running nooses which were applied on the forelegs of the already secured calf and which were not removed at any stage, were now tightened and handed to my assistant. Introducing both hands into the womb and guiding the gradual drag, I successfully delivered the rest of the foetus. The second calf died within a few minutes of delivery.

An antiseptic emulsion was applied into the womb and the placenta was removed with ease, but as the cow was straining considerably I feared an eversion of the womb and applied a rope truss.

I left my assistant to keep a careful watch for six hours and visited the case in the evening, when I repeated the penicillin injection and after examination found that the mother was doing well. She was under my treatment for 15 days and is none the worse for the operation. It is now February 1st, 1952, a month since the date of the operation, and she is quite normal and even the milk supply has not been affected.

The whole operation took two hours and 50 minutes.

The foetus, by a process of taxidermy, has since been preserved in its entirety and is now an exhibit in the Biological Section of the Colombo Museum.



A case of embryotomy of Siamese twins in a buffalo cow (Thoratti-Baroda).

DEEP-FREEZE STORAGE OF SPERMATOZOA

Joint Cambridge and Pretoria Universities Cattle Breeding Tests

Cambridge University and Pretoria University are to collaborate in scientific research which may lead to big changes in cattle breeding in South Africa, according to Professor F. N. Bonsma, Director of Animal Husbandry, who returned to the Union recently from Europe.

The research, says *The Times*, has the blessing of the British Ministry of Agriculture and the Union Department of Agriculture. It concerns the birth of calves at the Pretoria University research farm whose sires remain in England and whose dams have never left Pretoria. The limitation of artificial breeding all over the world to small, compact areas is expected to be overcome by a deep-freezing process for the storage of spermatozoa devised by Mr. Polge and Mr. L. E. A. Rowson, M.R.C.V.S., of Cambridge.

Cattle breeding experiments are to be started at Pretoria University's farm with deep-frozen material sent from England. Exports of this kind, which would enable calves anywhere to be sired by the finest bulls in Britain, are forbidden. But a special exemption for experimental purposes has been made in the case of Pretoria University.

Professor Bonsma said that the Cambridge discovery probably portended the greatest revolution in stock breeding of the century.

ABSTRACT

The Nutrition of the Young Ayrshire Calf*

In three further instalments of their series of papers on Ayrshire calves the Hannah Dairy Research Institute workers, with histological assistance from Glasgow University, deal with the problem of muscular dystrophy, cases of which they have encountered in the field as well as experimentally.

8. *Muscular Dystrophy in the Growing Calf.*—In experiments on nitrogen metabolism the sole source of vitamin E was a high-quality dried skim milk that had been packed in sealed tins and was believed to be adequate for the purpose. One of the experiments had to be abandoned, however, owing to the onset in six calves of a syndrome in which vitamin E deficiency appeared to be involved.

The calves in question were from 24 to 60 days of age. They became very weak and were unable to stand for more than a few minutes. The pastern joints became straightened and the hind legs crossed when walking was attempted. There was a general ataxia and the neck was outstretched in a characteristic way. During the later stages there was a tetany not involving the extremities, and seen only when the animal was placed on its feet. Relaxation of the throat musculature and inability to control tongue movements rendered drinking difficult, and in the terminal stages the calves choked owing to the entrance of liquids into the trachea. There was loss of hair at the bulbal base of the ear, the coat became staring and the skin thickened. In two calves oedema of the brisket and ascites developed suddenly, and was associated with slight exophthalmos and erratic pulse. These cases of apparent cardiac involvement died suddenly whereas the remainder died following a period of prostration. On *post-mortem* examination the carcase was generally slightly oedematous and rapid putrefaction was a constant feature. In some calves the heart was dilated and in one there were small white lesions on the wall of the left ventricle. The skeletal muscle was pale and fleshy and the fat was light brown (compared with the normal whitish-yellow). *Ante-mortem* serum examinations revealed normal values for Ca, Mg and inorganic P, and there was no response to the intravenous injection of magnesium sulphate. The urinary excretion of vitamin B_1 was not affected, and the only biochemical abnormalities detected were (*a*) an elevation of the urinary creatine from normal values of from 200 to 300 mg. per 24 hours to levels of up to 1,300 mg. per 24 hours ; and (*b*) an elevation of basal O_2 consumption by 20 to 60 per cent.

A diagnosis of avitaminosis E was made and one affected animal was treated successfully. There was no response to the daily administration of 25 mg. DL-alpha-tocopherol, but improvement rapidly followed daily dosage for five days with an aqueous solution containing 100 mg. A second calf, in which there was an acute alimentary disturbance and a pneumonia that did not respond to sulphonamide therapy, failed to benefit from the vitamin E.

Specific experiments were therefore set up in order to investigate the relationship of vitamin E intake to muscular dystrophy. A basal diet comprising dried skim milk, lard and a mineral supplement, when fed unsupplemented to Ayrshire calves initially two to three days of age, led to dystrophy similar to that described. Control calves receiving 50 mg. DL-alpha-tocopherol acetate remained normal, but in other calves the protective action of this level of vitamin E was neutralised by the daily ingestion of 15 to 18 ml. of cod-liver oil. The administration of cod-liver oil resulted, in fact, in a severe exacerbation of dystrophy symptoms, and it is concluded that the syndrome of cod-liver oil poisoning of calves described by Scandinavian workers represents a conditioned vitamin E deficiency.

There was a variation in the time taken for the individual experimental animals to exhibit the first signs of dystrophy ranging from about 21 days to over 40 days. Dystrophic calves stored less nitrogen than normal, there being an increased urinary excretion of nola and ammonia. The O_2 consumption was increased, and it is assumed that the excessive nitrogen catabolism and the increased heat production were probably interrelated.

9. *Composition of the Tissues of Normal and Dystrophic Calves.*—The dry-matter content of dystrophic muscles was lower than that of normal muscles but their ash-content was greatly increased and in the most seriously affected cases contained up to three times the normal amount. This increase was in part attributable to calcification of the dystrophic muscles, the Na content of which was also increased. The P content was slightly raised, but the K and Fe contents were decreased while Mg values were within the normal range.

On a dry-matter basis the total lipid content of dystrophic muscle was higher than normal, whereas the total N content was lower. There was an increase in collagen N and in stromaprotein N, but a marked decrease in globulin N. The nucleic acid content was increased.

There was a marked reduction in the creatine content of dystrophic muscles and this reduction was bilaterally symmetrical in the individual calf. The classification of affected muscles by visual examination agreed only partly with their creatine content.

The brains and the livers of calves deprived of vitamin E had lowered dry-matter contents.

It is considered that the results can be explained on the basis of a major change in the synthesis or destruction, or both, of globulins in dystrophic musculature.

10. *Histopathology of Muscular Dystrophy and its Relation to Muscle Chemistry.*—The histological features of the lesions of dystrophic calves are tabulated and it is shown that they agree well with those described in vitamin E deficiency in other species. There appear, however, to be three discrepancies. First, the muscle cells in the calf retain their striated appearance despite their considerable fragmentation. Second, there is massive sarcolemmal proliferation. It is suggested by the authors that this may be an attempt to maintain constant the volume of dystrophic muscles, and may reflect the fact that at this stage of development a young calf would store large amounts of protein but negligible quantities of fat. (In other species it would appear that fat is deposited in an attempt to maintain muscle volume.) The third point of difference is the presence of calcification.

A. N. W.

* **The Nutrition of the Young Ayrshire Calf. 8.—Muscular Dystrophy in the Growing Calf ; 9.—Composition of the Tissues of Normal and Dystrophic Calves ; 10.—Histopathology of Muscular Dystrophy and its relation to Muscle Chemistry.** 8 and 9, BLAXTER, K. L., *et al.*; 10, MACDONALD, A. M. (1952.) *Brit. J. Nutrit.* **6.** 2, 125–169.

MILK-BORNE DYSENTERY OUTBREAK IN SOMERSET

Infected milk from a cow in a tuberculin tested herd near Chard is thought to have caused an outbreak of dysentery among more than 600 schoolchildren and adults. She was seen to be off colour and was isolated a few days before the outbreak occurred; eventually salmonella infection was confirmed and she was slaughtered.

REVIEW

[The Inspection of Food: A Handbook for Students of Public Health, Agriculture and Meat Technology. By H. Thornton. viii + 223 pp., with 58 figures. Baillière, Tindall & Cox, Ltd., London. 1952. Price 12s. 6d.]

This work has been prepared in the main as a "primer" for students in the meat trade taking the examination in the "science of meat and meat inspection," and for sanitary inspectors taking the examination in "the inspection of meat and other foods" of the Royal Sanitary Institute.

An attempt has been made to compress into 200 pages of a book which can be slipped into the pocket, the essential elements of veterinary anatomy, physiology and pathology and food inspection and is hardly likely to have an appeal to veterinary readers. The sections in the book which deal with fish and foods, other than meat and milk, however, could well be used by veterinary students as an elementary introduction to those aspects of food inspection of which, although they are of minor interest to them, some superficial knowledge is desirable. To those veterinary surgeons who, for the first time, may be planning a course of lectures to lay persons interested in food inspection, this book might be of assistance in the drawing up of headings for lectures but less so as a source of material. The information contained in the handbook will no doubt suffice for students in the meat trade who are taking the Craftsman's and Ordinary Certificate examinations of the Institute of Meat, but it is doubtful if it is sufficiently detailed for the Royal Sanitary Institute's examinations in food hygiene or food inspection; nor is it likely to be of much use to agricultural students who will, no doubt, prefer to invest in a text-book specifically written to cover their requirements.

The reviewer, who fully appreciates the obvious painstaking care and research which must have been lavished on Thornton's "Textbook of Meat Inspection," cannot escape the conclusion that the task of preparing this handbook has not been undertaken with the same care, with a result that does not do credit to the author of the text-book. This criticism relates mainly to that part of the book which deals with meat inspection; the sections which deal briefly with the other foods are, it is felt, less open to question and indeed many are very well prepared and present in a concise manner all the essential details necessary to the food inspector. The work is well illustrated by clear and well-drawn diagrams which, it is felt, may be of greater use to the student than much of the text, which sometimes leaves one in doubt as to the action which a food inspector should take when he meets with certain of the conditions mentioned in the handbook. One is occasionally inclined to feel that the text-book and handbook are not entirely in agreement on some points.

One can sympathise with the author (notwithstanding the fact that he was actively engaged as a local authority inspector under the Diseases of Animals Act) in his apparent inability to interpret, into simple language, the intentions of some of the Orders made by the Ministry of Agriculture, but it is difficult to understand how he has introduced the name of a country (Yugoslavia) which has never appeared in any of the Poultry Carcases (Importation) Orders made on, or subsequent to, June 22nd, 1950. As the reviewer understands the position, at the moment of writing (August, 1952), the importation of uncooked poultry carcases is prohibited from all countries except the whole of Ireland, Jersey, Australasia, Argentina, Uruguay and Southern Rhodesia and also, unless accompanied by a specified certificate, from Norway, Sweden and Denmark.

REPORT

[Report of the Medical Research Council for the Year 1950-1951. Pp. 214. Published by H.M.S.O., London. Price 6s. net.]

The report for the year 1950-51 again records substantial progress in researches not only on problems directly concerned with human health but also on a variety of allied subjects which have an indirect bearing on the elucidation of diseases of man.

The Medical Research Council has for some time past been concerned with the training of research workers. It recognised that the main responsibility falls on universities and has awarded many grants to provide for the training of junior workers. The arrangements for suitable post-graduate instruction have recently been under review. There are now three main schemes: post-graduate scholarships, clinical fellowships and travelling fellowships. It may be noted that whilst the majority of the awards went to medical men and women, a number were granted to graduates in the natural sciences: over one half chose to work in subjects allied to chemistry whilst only one quarter selected the group anatomy, physiology and pharmacology. It is impossible to review all the work sponsored by the Council and, as has been done on previous occasions, points have been selected which seem to be of direct interest to veterinarians.

Considerable advances have been made in the preservation of living cells at low temperatures and it has now been shown that tissues may survive freezing in "dry ice" or in liquid air. The earlier work with bull spermatozoa demonstrated that the method of quick freezing in glycerol, which was most satisfactory with fowl spermatozoa, was not applicable but further experiments have shown that slow cooling in glycerol was compatible with the revival of a large percentage of the cells. A series of trials carried out at Cambridge have resulted in a pregnancy rate of nearly 80 per cent. after insemination with semen stored at $-79°$ C. from periods varying from two hours to eight days. This method is also of value in the preservation of red cells. A technique has now been developed for an aseptic method for freezing, thawing and dialising batches of human blood large enough to permit transfusion with the recovered cells.

The investigations into infantile gastro-enteritis, which have been under the control of one of the Council's committees for several years, are still making progress. Recently in 42 out of 44 cases of acute summer diarrhoea in infants a particular type of coliform organism has been found. In a series of 100 control infants it was recovered in four cases only. Subsequent investigations into institutional outbreaks of the disease have indicated that this so-called "alpha" type is to be found in practically every affected child. It is too early to say definitely whether this coliform organism is responsible for this form of infantile gastro-enteritis. On the assumption that the "alpha" type plays some part and bearing in mind that it is susceptible to some of the newer chemotherapeutic agents, clinical trials are being undertaken in a number of centres.

Concluded on page 628

Trials on the prevention and treatment of tuberculosis have again been in the forefront of the Council's investigations. A national survey of the tuberculin sensitivity of 95,000 young people has been completed as a preliminary step for the estimation of the results of the immunising efficacy of B.C.G. and vole bacillus vaccines mentioned in the last report. Great interest has been aroused in the results of preliminary clinical trials with two new products, iso-nicotinic acid hydrazide and its isopropyl derivative, and laboratory experiments as well as preliminary clinical trials are now under way in various institutes controlled by the Council's tuberculosis research unit.

During the years 1948 to 1950 three new antibiotics, chloramphenicol, aureomycin and terramycin, which have greatly extended the range of diseases amenable to chemotherapy, have been brought into use in this country. It is pointed out that in respect of the use of these new drugs American experience is several years ahead of that in this country. The main properties of all three drugs are now fairly well known. They appear to be specific for all forms of typhus and other infections due to rickettsiae and to small organisms intermediate in size between bacteria and viruses. It is evident that these substances are worthy of assay in certain of the diseases in the veterinary field which are caused by viruses in this particular group. They are particularly valuable in many of the bacterial infections because they may be active against organisms which have developed a resistance against penicillin or streptomycin. These dugs are also being used against certain forms of pneumonia, especially those which are attributed to virus infections. A note of caution, however, is sounded and it is pointed out that the initial enthusiasm over a new drug must be tempered with caution since carefully controlled trials often fail to support the favourable clinical impressions which are advanced when these drugs first come into use.

Other investigations are concerned with diseases of the eye, skin diseases, climatological medicine—which includes the effects of heat and cold and acclimatisation, antimalarial drugs and insecticides—the various chemical agents which may be responsible for cancer, and the action of cortisone and allied substances in the treatment of a variety of conditions.

The report is completed by a useful appendix in which is reproduced a memorandum prepared by Dr. Addison, Minister of Health in 1918, in which the organisation of the Medical Research Council is outlined. It is pointed out that after a lapse of 20 years the scheme which he advocated still seems to give the best results and to provide full scope for unhampered research in all branches of medicine.

R. E. G.

DANGER OF "EXCESSIVE MEDICATION"

Professor David Campbell, President of the General Medical Council, in his charter inaugural address at the opening of the 111th session of the University of London School of Pharmacy on October 8th, said that prescribing in the National Health Service should be confined to drugs and preparations made official in the pharmacopoeia.

The danger now, he said, was excessive and inaccurate medication, and that danger was not lessened by persistent advertisement in the form of the quasi-scientific and clinical dissertations which were showered so freely on the medical profession by manufacturing drug firms. These were often the same drug under half a dozen or so different proprietary names.

NOTES AND NEWS

Diary of Events

Oct. 22nd.—Meeting of the Lancashire Division, B.V.A., at Liverpool, 2.30 p.m.

Oct. 22nd.—Meeting of the Scottish Metropolitan Division, B.V.A., at the Ministry of Agriculture Laboratory, Lasswade, 2.30 p.m.

Oct. 23rd.—Annual Dinner and Dance of the Midland Counties Division, B.V.A., at Birmingham (Midland Hotel); Reception, 7.30 p.m.

Oct. 24th.—Meeting of the Biochemical Society at St. Thomas's Hospital Medical School, 2 p.m.

Oct. 24th.—Meeting of the Society for the Study of Animal Breeding, at the Wellcome Research Institute, 183-193, Euston Road, London, N.W.1, 2.15 p.m.

Oct. 28th.—Meeting of the South Eastern Division, B.V.A., at Maidstone (Royal Star Hotel), 6.30 p.m.

Oct. 30th.—Joint Meeting of the Sussex Division, B.V.A., and the Brighton Division, B.M.A., at Brighton (Hotel Metropole), 8.30 p.m.

Oct. 31st.—Autumn Meeting of the Eastern Counties Division, B.V.A., in the Department of Animal Pathology, Milton Road, Cambridge, 12 noon.

B.V.A. Committee and Council Meetings

It has been decided to hold the next Committee and Council Meetings of the B.V.A. on Wednesday, Thursday and Friday, November 5th, 6th and 7th, 1952, in the following order:—

Wednesday, November 5th:
12 noon. Parliamentary and Public Relations Committee.
2.30 p.m. Veterinary State Medicine Committee.

Thursday, November 6th:
10.0 a.m. Organising Committee.
12 noon. Home Appointments Committee.
2.15 p.m. General Purposes and Finance Committee.

The above meetings will be held at 36, Gordon Square, London, W.C.1.

Friday, November 7th:
11.0 a.m. Meeting of Council at the Connaught Rooms, Great Queen Street, W.C.2.

Nov. 6th.—Meeting of the Society of Women Veterinary Surgeons, at the Royal Veterinary College, Camden Town, 2.45 p.m.

Nov. 6th.—Joint Meeting of the Central Division, B.V.A., and the Society of Women Veterinary Surgeons, at the Royal Veterinary College, Camden Town, 6 p.m.

Nov. 12th.—R.S.I. London Sessional Meeting: Benjamin Ward Richardson Lecture on the hygienic and economic utilisation of offal and by-products of food animals, by H. E. Bywater, M.R.C.V.S., Chief Veterinary Officer, West Ham.

Nov. 20th and 21st.—B.V.A. Conference on Metabolic Disorders and Other Problems Related to Grassland and Fodder Crops and Innovations in Animal Husbandry, to be held in the Auditorium of the Wellcome Foundation, 183, Euston Road, London.

R.C.V.S. Examinations

Animal Management Examination.—Tour commences with London on Friday, November 7th, 1952.

Pharmacology Examination (Revised Syllabus).—Written: Friday, November 7th, 1952. Orals and Practicals: Commencing November 10th or 11th.

Membership Examinations.—Written: Thursday and Friday, December 4th and 5th, 1952. Orals and Practicals: Commencing Monday, December 8th, 1952.

B.V.A. President's Visit to Denmark

At the invitation of the Danish Veterinary Association the President of the British Veterinary Association (Mr. A. M. Graham) attended the annual Congress of that Association as the official representative of the B.V.A. and he left England on Friday of last week to spend the week-end in Copenhagen.

With their well-known hospitality, Mr. Graham's Danish veterinary colleagues were waiting to welcome him when he arrived at his hotel after a smooth flight, Dr. and Fru Seidel, particularly, looking after him that evening.

The next day, Saturday, Mr. Graham met Denmark's Chief Veterinary Officer, and also Dr. Koch, President, and other officers of the Danish Veterinary Association. He was entertained to lunch and dinner on that day and shown over the Veterinary College and the Ministry of Agriculture headquarters, where he learned something of the various schemes on foot in connection with animal health.

On Sunday—the day of the Danish Veterinary Congress—Mr. Graham first attended the annual general meeting and was officially welcomed there. There was a paper on mastitis in the afternoon and, later, Mr. Graham was taken on a tour of the lovely City of Copenhagen.

The visit culminated in the annual banquet on Sunday evening. The President of the Swedish Veterinary Association and the Vice-President of the Norwegian Veterinary Association were there together with a company of about 100 and everyone had a very happy evening.

Mr. Graham reports that he had a very enjoyable time indeed during his week-end abroad and feels that his visit was well worth while.

* * * * *

DEPARTMENTAL COMMITTEE ON FOOT-AND-MOUTH DISEASE

The Ministry of Agriculture announces that the Departmental Committee recently appointed by the Minister of Agriculture and Fisheries to review the policy and arrangements for dealing with foot-and-mouth disease in Great Britain held its first meeting on Thursday of last week. Memoranda of evidence are being submitted to the Committee by the British Veterinary Association and by the Royal College of Veterinary Surgeons, who were among the bodies invited to offer evidence in that form.

* * * * *

PERSONAL

Mr. E. C. Lloyd's Appointment with FAO

Mr. E. C. Lloyd, C.B.E., M.R.C.V.S., Senior Deputy Chief Veterinary Officer of the Ministry of Agriculture and Fisheries until his resignation in June last, has, on the invitation of the Food and Agriculture Organisation of the United Nations, recently completed a preliminary visit to Yugoslavia as a consultant on the control of animal disease. He has now joined the staff of FAO and has returned to Belgrade via Rome in order to advise and assist the Yugoslav Government in the development of an animal disease field control programme.

. . . . .

Award of R.C.V.S. Fellowship.—On September 30th, 1952, Mr. David Leslie Hughes, M.R.C.V.S., DIP. BACT. (LOND.), of the Veterinary Science Division, Boots Pure Drug Co., Ltd., Nottingham, was awarded the Fellowship Diploma of the Royal College of Veterinary Surgeons. The subject of Mr. Hughes's successful thesis was "Studies of methods for controlling the spread of *Str. agalactiae* in dairy herds."

. . . . .

Forthcoming Marriage.—HENDERSON—JAGGER.—The engagement is announced between Gordon N. Henderson, B.SC. (EDIN.), M.R.C.V.S., of Guildford, and Sheila Jagger, of Godalming, Surrey.

* * * * *

Births.—ARNOLD.—On September 20th, 1952, at 107, Northampton Road, Kettering, to Megan and Geoffrey Arnold, a second son.

DOUGAL.—On October 9th, 1952, at Fort House Nursing Home, Dundee, to Claire (Hollingsworth), wife of Laurence Dougal, M.R.C.V.S., Craignish, Pittenweem, Fife, a son—Gavin Laurence.

JONES.—October 13th, 1952, at the County Hospital, Bangor, to Pearl, wife of Eric B. Jones, B.V.SC., M.R.C.V.S., a son—brother for Margaret and Daphne.

TULLOCH.—On October 5th, 1952, to Ray and Inglis, at "Westhill," West Street, Ryde, Isle of Wight, a son.

WILSON—*née* Benham.—On September 13th, 1952, a daughter was born to Cicely Wilson, M.R.C.V.S., wife of Arthur Wilson.

* * * * *

R.C.V.S. OBITUARY

BECK, D'Arcy Stuart, Major R.A.V.C. (retd.). Died on October 9th, 1952, at Cherry Trees, Wilkie Road, Birchington.

CREAGH, Pierce Cornelius William, Captain R.A.V.C. (retd.), of Kilmahon, Shanagarry, Co. Cork, Eire. Graduated Dublin, July 20th, 1925. Died October 5th, 1952.

HOLMES, Roger Heuston, C.M.G., F.R.C.V.S., Colonel A.V.S. (retd.), of Carnone, Tipperary, Eire. Graduated Edinburgh, May 26th, 1893; Fellowship December 8th, 1906. Died October 4th, 1952; aged 81 years.

WILLIAMS, John Llewellyn, M.R.C.V.S., D.V.H., Captain late R.A.V.C., of Awelon, Bala. Graduated London, December 22nd, 1914. Died October 10th, 1952.

Captain J. L. Williams was one of the older members of the North Wales Division, B.V.A., and we hope to publish an appreciation in our next issue.

WILSON, John Mounsey, B.SC. (EDIN.), M.R.C.V.S., Beech Cottage, 18, Victoria Road, Harrogate, Yorks. Graduated Edinburgh July 9th, 1947. Died October 10th, 1952; aged 29 years.

. . . . .

Colonel R. H. Holmes, C.M.G., F.R.C.V.S.

Colonel Roger Heuston Holmes, C.M.G., F.R.C.V.S., whose death we record above with deep regret, was the son of the late Rev. J. Holmes, of The Manse, Tipperary. He was educated at The Abbey, Tipperary, and took his diploma as a member of the Royal College of Veterinary Surgeons at the Edinburgh College in 1893, securing his Fellowship in 1906. He was with the Tirah Expeditionary Force in 1897, and during the first world war he was an Assistant Director of Army Veterinary Services. Colonel Holmes, who served in France, was twice mentioned in despatches and was awarded the C.M.G. Later, he was appointed Deputy Director of Veterinary Services in the Irish Command.

He was interested in various benevolent activities in Tipperary, and was a member of Tipperary Vocational Education Committee.

. . . . . .

John Mounsey Wilson, B.Sc., M.R.C.V.S.

AN APPRECIATION

The news of the tragic death of "Johnny" Wilson will be heard with great sorrow by all men who had the pleasure and privilege of knowing him. He was a student at the Royal (Dick) Veterinary College from 1942 to 1947, when he qualified. He was a brilliant scholar, and would have excelled in whatever branch of the profession he chose to enter. In his case, this was general practice, and he was assistant to Mr. Cubby, of Dalston, and Mr. Rorrison, of Derby. During his second assistantship, he contracted a serious kidney disease and was in hospital for many months. Against all predictions, he made an apparently complete recovery. In July, 1951, he married Miss Margaret Tulloch, of Doncaster, who had been at the Edinburgh Domestic Science College while John Wilson was studying at the "Dick." Together, they set up both home and practice in Harrogate.

The winter, however, brought a relapse of the old condition and, although he knew his end was very near, and despite changes of a permanent character affecting his sight, he continued to practise. He was indomitable. Extensive oedema set in, but he still struggled to the surgery to see his clients. In June, his last words before I drove away were, "Well, Pat, I hope to see you soon, if I am still for this world." He had the fluid drained from his body and attended the Congress as though nothing were wrong. In October he has died. I would like to make these facts known, for I think very few are aware of them, and such courage should be made known to all. It can truly be said here that if this world is poorer by his death it is tremendously richer for the way he faced it.

All who knew him will extend their deep sympathy to his wife, to his mother, and to his brother.

P. S. L.

XUM

Harold Stainton

A Further Tribute

Mr. L. R. Geffen, M.R.C.V.S., writes from Jamaica, B.W.I.: "May I add a few remarks of appreciation to those that already have appeared. I 'gate-crashed' into Kensington in 1942 and put up a brass plate not far away from Stainton's practice. For nine years we were cheerful rivals: patients of his came to me and mine went to him, yet at no time did we ever cross swords or have an angry word. Whenever I needed the help of a good friend Harold Stainton gave me freely of his time and advice. His thoroughness, his thoughtfulness and his kindness knew no bounds.

"He was a man of many parts, a great humorist and a great gentleman. There are still a few of the 'Old Guard' left in the profession, but the mould from which they were cast has been broken and we will not look upon their like again. Such a man was Harold Stainton. His death is a loss that will be felt, not only by his family and his many friends, but throughout the entire profession."

* * * * *

R.A.V.C. DIVISION VISITS THE A.H.T. EQUINE RESEARCH STATION

For its third meeting (the proceedings of which will be reported in our supplement in due course) the R.A.V.C. Division of the Association enjoyed a particularly memorable programme in a visit to the Animal Health Trust's Equine Research Station at Newmarket on September 24th. Under the guidance of Professor Miller, Colonel Barnett, Lieut.-Colonel Hickman and Dr. Farrelly, a tour was made of the premises at Balaton Lodge. Following an adjournment for lunch, the members reassembled in the lecture room at Lanwades Park, where the Chairman, Brigadier Heveningham (Director, Army Veterinary and Remount Services), reopened the meeting for the reading and discussion of papers by Professor Miller, Colonel Hickman and Dr. Farrelly. A tour of Lanwades Park followed, with tea.

* * * * *

ADDRESSES OF DISEASE-INFECTED PREMISES

The list given below indicates, first, the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax:

Aberdeenshire.—Dunecht House Farm, Culfosie, Echt, Aberdeen (Oct. 9th).

Berks.—Bridge Farm, Arborfield, Reading (Oct. 10th).

Cheshire.—Somerford Farm, Somerford, Congleton (Oct. 7th).

Devonshire.—Ashcombe Barton, Ashcombe, Dawlish (Oct. 8th).

Dunbarton.—Whiteless Farm, Castlecary (Oct. 8th).

Essex.—Moor's Farm, Assington, Colchester (Oct. 6th).

Hants.—Ashley Manor Farm, Ashley, New Milton (Oct. 6th).

Herts.—The Folly, Baldock Road, Buntingford (Oct. 3rd).

Kent.—Nightingale Farms, Southborough, Tunbridge Wells (Oct. 4th); North Court Farm, Tilmanstone, Sandwich; Little Poplar Farm, Headcorn, Ashford (Oct. 6th); "Fairbank," Smeeth, Ashford (Oct. 8th); Collinette Farm, Brasted, Sevenoaks (Oct. 9th).

Lancs.—Higher Green Head Farm, Sawley, Clitheroe; Longworth Farm, Ramsgreave, Blackburn (Oct. 6th); Paa Farm, Paythorn, Gisburn, Clitheroe (Oct. 7th); Parsonage Farm, Chipping, Preston (Oct. 8th).

Leics.—Oaklands Farm, Shackerstone, Nuneaton; Northfield Farm, Northfield Road, Blaby, Leicester (Oct. 9th).

Monmouthshire.—Black Rock Farm, Portskewett, Chepstow (Oct. 4th).

Norfolk.—Hall Farm, Stratton Strawless, Norwich (Oct. 3rd).

Somerset.—Court Farm, Markesbury, Bath (Oct. 4th); Pearce's Field, Ashbottom, Tintinhull, Yeovil (Oct. 6th); Manor Farm, Stone Easton, Bath (Oct. 7th).

Staffs.—Salt Lodge Farm, Sandon, Stafford (Oct. 3rd); The Paddocks Farm, Keele, Stoke-on-Trent (Oct. 6th).

Sussex.—Legsheath Farm, Forest Row (Oct. 3rd).

Wilts.—Southgrove Farm, Burbage, Marlborough (Oct. 3rd); Stroud's Farm, Chisbury, Marlborough (Oct. 4th); Manor Farm, Patney, Devizes; Smithcot Farm, Dauntsey, Chippenham (Oct. 6th).

Yorks.—Sportsman's Arms Hotel, Wath, Pateley Bridge, Harrogate; West Shelding Farm, Grantley, Ripon (Oct. 4th); Shepherd's Lodge Farm, Hartwith, Ripley, Harrogate; Sheep Ings Farm, Lower Hopton, Mirfield (Oct. 6th); Hollins Farm, High Birstwith, Harrogate (Oct. 7th).

Fowl Pest:

Essex.—4, Bridge Terrace, Stratford, London, E.15 (Oct. 6th); The Hedges, Leslie Road, Rayleigh (Oct. 8th).

Herefordshire.—Buckland, Bartestree, Hereford (Oct. 8th).

Norfolk.—Spring Cottage, Great Dunham, King's Lynn (Oct. 3rd); Hall Farm, Hingham, Norwich (Oct. 7th).

Sussex.—Ovingdean Hall Farm, Ovingdean, Brighton (Oct. 8th).

Swine Fever:

Cambs.—Randswood Farm, West Wratting, Cambridge (Oct. 6th).

Denbighs.—Pennant Hall Farm, Eglwysbach, Talycafn (Oct. 7th); Tyn-y-Bryn Farm, Eglwysbach, Talycafn (Oct. 10th).

Flints.—Carr Road Allotments, Felixstowe; Cefendy Farm, Cefendy Road, Rhyl (Oct. 9th).

Kent.—Lucky Wood Allotments, Crabble Close, River, Dover (Oct. 4th).

Lancs.—Edenfield Farm, Goosnargh, Preston (Oct. 6th).

Lincs.—Bratoft, Skegness (Oct. 6th); R.A.F. Station, Scampton, Lincoln (Oct. 7th); Manor Farm, Revesby, Boston (Oct. 11th).

Middlesex.—2, Smallholdings, Bedford Lane, Feltham (Oct. 4th); 6, Bedfont Court, Spout Lane, Stanwell, Staines; Clock House Nurseries, Forty Hill, Enfield; 158, Staines Road, Sunbury-on-Thames (Oct. 6th).

Northants.—320, Longthorpe, Peterborough (Oct. 3rd).

Oxon.—Lower Farm, Mollington, Banbury (Oct. 6th).

Suffolk.—Sharpe's Corner, Lakenheath, Brandon (Oct. 3rd).

Warwicks.—Tile Barn Farm, Farnborough, nr. Kineton (Oct. 11th).

Worcs.—Mill Bank Farm, Oldbury Road, St. John's, Worcester (Oct. 7th).

* * * * *

COST OF GERMAN PERIODICALS

German periodical publications, unavailable during the war, have again become an expensive item in the annual budget of university and other learned libraries, states *Nature* in comment on an article in that journal, September 13th, page 446, by Dr. Wilfred Bonser, Librarian, University of Birmingham, and Miss M. P. Russell, Deputy Librarian of the Medical School. By a price analysis of a selection of representative medical journals, these authors have shown that the cost of German journals alone exceeds the combined sum expended on those from other countries. This, in spite of the fact that increases on account of the devaluation of sterling amounted to 45 per cent. for American, and only 26 per cent. for German periodicals.

In 1933, when a previous investigation was undertaken, the average price ratio for scientific periodicals was British, 1·0; American, 1·06; other countries, 0·875; German, 3·8. In 1950-51, the average ratio was: British, 1·0; American, 1·69; other countries, 0·96; German, 4·15. Although a reduction in price of 20 per cent. was obtained in 1933, and a further reduction of 25 per cent. in 1935, pre-war complaints were not only as to the excessive cost, compared with that of British periodicals of a similar nature and size, but also as to the impossibility of computing the annual expenditure because of irregularity in the number of volumes per year. The same state of affairs, say the authors, still obtains. Although it has been stated that German journals contain more illustrative material, making them more costly, a comparison, for instance, of the *British Journal of Experimental Pathology* with the *Archiv für experimentelle Pathologie* in respect of pages, plates, photographs, line drawings and tables, lends little support to this contention. The Germans have promised to co-operate in an effort to reduce the excessive price of their journals, but, the present situation whereby circulation is curtailed on account of prohibitive costs, is, say the writers, to be deplored.

CORRESPONDENCE

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A

ANTIBIOTICS IN ANIMAL NUTRITION

Sir,—With reference to the short note on antibiotics in animal nutrition which appeared in *The Veterinary Record* of September 27th, our experience here may be of interest. During the past three years many experiments have been carried out mostly with turkeys and a smaller number with chickens. A vast amount of data has accumulated but pressure of routine work has prevented publication, except for a short note in *Nature* of April 26th, 1952, when the results of some preliminary experiments were reported. Invariably, there has been a marked increase in growth rate in groups receiving penicillin (12 grammes to the ton) compared with control birds kept under identical conditions, provided such conditions approximated to those of a poultry farm; this proviso is of great importance.

In preliminary field experiments phenomenal results were obtained in certain groups but it was found that under laboratory conditions the response was always much less. Later, groups of turkeys and chicks were placed in brooders, cleaned but not sterilised after previous use within a house in which other brooders containing turkeys and chicks were also present. Controls were kept in similar brooders which had been sterilised, in an air-conditioned room away from contact with poultry. There was no significant difference between the groups with and without penicillin in this environment and those receiving penicillin under the exposed conditions, but there was a marked difference between the latter group and that without penicillin.

It would appear, therefore, that the better the conditions the less is the effect of the antibiotic supplement and that under what is virtually a "perfect" environment no response is obtained. On even the best-run premises, particularly under modern methods when large groups of young chicks and poults are passing through the brooder house each week, conditions can hardly be expected to reach, and certainly not maintain, such a standard. It would appear, then, that in practice the addition of penicillin to the diet is worth while.

When antibiotic supplements are fed to day-old poults on the farm the response is well marked within a fortnight and even more so in a month; as previously stated, the difference in weights may be phenomenal. As birds become older this variation is less marked and at killing time it may be as little as 1 lb.

In our experience an even more striking feature was the effect on non-specific mortality, particularly in turkeys. This was most obvious under field conditions, less noticeable under laboratory conditions and absent in the groups under perfect "conditions." In some cases where there was a mortality of 25 per cent. in control groups of young poults during the first few weeks of life, the death rate in the treated groups was insignificant. This happened so consistently that any possibility of chance may be ruled out.

Growth rate and mortality during rearing are factors of primary economic importance to the turkey industry and largely determine the profitability of the undertaking. The beneficial effects of the addition of penicillin supplement to the starter mash suggest that its more general use would be justified. There may or may not be valid objections to feeding it to future breeding stock; there would appear to be nothing against its use for birds destined for the table within the shortest possible time.—Yours faithfully, J. E. Wilson. Ministry of Agriculture and Fisheries, Veterinary Laboratory, Eskgrove, Lasswade, Midlothian. *September 30th*, 1952.

* * * * *

TREATMENT OF PAPILLOMATA

Sir,—Messrs. Sumner and Crawshaw have recorded good results in the treatment of multiple papillomata in the bovine with "Anthiomaline," and I wish to describe the successful treatment of a papilloma on the glans penis of a two-year-old Ayrshire bull.

An attempt was made at surgical removal and the walnut-sized superficial part of the growth was removed. However, it was found impossible to remove the entire growth as several parts were deeply embedded in the penis. These "roots" were seen to be isolated from one another and affected a considerable part of the glans. Within two weeks the papilloma had regrown to its original size.

"Anthiomaline" treatment was decided upon and 100 c.c. in 10 c.c. doses were injected over a period of five weeks. At the end of this time the growth was re-examined and found hanging from the penis by a mere thread-like strand. The papilloma was very easily removed.

It is felt that the doses given in this case (similar to the manufacturer's recommendation) may be more suitable for the treatment of such deeply embedded papillomata than the shorter courses of treatment previously described.—Yours faithfully, A. Fraser, Paragon House, Stanhope, Co. Durham. *October 10th*, 1952.

* * * * *

USE OF CHLOROPHYLL IN DOGS

Sir,—The article by Messrs. A. N. Worden and C. E. Waterhouse regarding the use of chlorophyll in dogs, which was published in your issue of September 27th, provided some interesting observations on this very topical subject.

Whilst their general results conform to much of the evidence which is accumulating in regard to halitosis and body odours, their experience with bitches in pro-oestrus and oestrus is somewhat surprising.

In my own experience, the daily administration to bitches weighing about 20 lb. of one tablet containing 65 mg. of a water soluble chlorophyll preparation, assaying 20 per cent. of sodium chlorophyllins, produced the desired result in some 20 out of 25 observed cases. Munsch, in a paper presented to the American Pharmaceutical Association this year on the "Present State of Chlorophyll," referred to the remarkable effect of chlorophyllins on the mating habits of bitches. He reported that the administration of about 25 mg. per day inhibited any response from male dogs.

One of the great difficulties in assessing the action of "chlorophyll" has been, and to a large extent still is, the lack of precise information about the type and purity of the material used. This, of course, involves the important question of dosage in terms of apparent active component. Worden and Waterhouse used a water-soluble chlorophyll stated to comply with the 1934 B.P.C. monograph. Such preparations are not standardised and are of very uncertain chemical composition and likely to be relatively crude in comparison with many of the more recently developed materials. The

authors made the point that their conclusions did not relate to the latter, but they state that they did, however, obtain substantially similar results with two proprietary brands so far as halitosis was concerned. They make no reference as to whether or not they were able to confirm their first findings with bitches in pro-oestrus and oestrus, and it would be unfortunate if their conclusions were to be interpreted as implying that water-soluble chlorophylls in general are ineffective for that purpose.

There are two important factors which must be observed if satisfactory results are to be obtained with bitches in pro-oestrus and oestrus. Firstly the dosage must be sufficient and secondly the tablets should be administered a few days prior to oestrus. If the latter has not been possible, then a double dose should be given for three or four days and the hind quarters thoroughly washed in some detergent deodorant, as chlorophyll will not dispose of extraneous odours.

In my experience over a period of nearly a year, adequate dosage of a standardised chlorophyllin given at the right time gives very satisfactory results in preventing dogs from being attracted to bitches in oestrus.—Yours faithfully, G. E. Joss, 6, Earlswood Road, Redhill, Surrey. *October 3rd*, 1952.

* * * * *

Sir,—It is with some interest that I have read the report of Messrs. A. N. Worden and C. E. Waterhouse, stating the results which they had obtained with the use of water-soluble chlorophyll.

It is not the purpose of my letter to comment on the tests which they carried out, although I feel these to be very incomplete, but merely to report briefly on my own findings which have differed considerably.

For some time I have been using a veterinary tablet consisting of a water-soluble U-V activated chlorophyll, and have tried its efficiency in a large number of conditions. These have included halitosis from gastric disturbances, various dental origins, ulceration of the gums, and labial eczema, and I have found almost complete de-odorisation as a result of the administration of the tablets two or three times daily.

The most remarkable results, however, have been obtained in the cases of bitches during the oestrous cycle. These tablets have been administered to six bitches under my direct personal supervision. In three cases the owners had both a dog and a bitch living in a normal household. No attempt was made to segregate the dog from the bitch in order that the behaviour of the animals could be watched. In none of these cases did the dogs show any interest in their partners although in one case the bitch (a golden cocker spaniel) made every effort to seduce the dog. The dosage was one tablet every 12 hours in each case.

In the case of the other three bitches they were taken for normal walks by their owners and were treated with no special care, and yet no dog showed any undue interest in them.

As these results are so directly opposed to those reported in the article it would be very interesting to hear the results of other members.—Yours faithfully, E. W. Fawcus, The Hackbridge Kennels, Hackbridge, Wallington, Surrey. *October 6th*, 1952.

* * * * *

AUTOGENOUS VACCINE THERAPY IN DISTEMPER AND HARD PAD

Sir,—I write you because I believe that many members of the profession will be interested in the results of an experiment recently conducted in a treatment for canine distemper and hard pad.

The treatment was used in two series of cases, one series treated by me at the R.S.P.C.A. Hospital at Putney, and the other series by W. C. L. Orr, B.SC., M.R.C.V.S., in his London practice.

The treatment was devised on the theory of Dr. W. M. Crofton that bacterial and virus activities are different phases of the same organism, the virus attacking internal tissues and fluids while the bacteria are found on surface membranes. Thus he believes that vaccines made from the surface bacteria prove effective against virus infections. He has proved this by complete filtration experiments in the original virus disease, foot-and-mouth disease and other hitherto so-called "virus" diseases.

Technique.—The culture medium—blood agar—contained in sterile screw cap bottles, was sown direct from each dog by sterile swab impregnated from pharynx and tonsils. The bottles were then sent in wooden containers to the laboratory and the vaccine received back usually within 72 hours. The vaccine was issued in 12 numbered vials containing increasing dosage to be administered as a series of injections at three- to four-day intervals. The injections were continued until all clinical symptoms had disappeared. No other form of treatment was given.

The majority of cases were diagnosed clinically as hard pad, although no differentiation between hard pad and distemper was required.

In both series, in every animal a gram-negative bacillus was isolated. This bacillus was indistinguishable from Copeman's distemper bacillus. In some cases gram-positive cocci were also present.

Cases Treated by W. C. L. Orr during the period 1949 to March, 1952

Treated	50 dogs
Recovered	42 „
Deaths or destructions	8 „
Recovery	84 per cent.

Of the eight cases that died or were destroyed, six cases were advanced, and most had severe fits or paralysis before treatment commenced. Thus in cases taken early enough, recovery rate was 96 per cent.

Ten of the 50 cases had been inoculated against distemper with the Field vaccine. The average number of injections given was 10.

The trial at the R.S.P.C.A. Hospital was run with one set of dogs receiving vaccine therapy and a second set, used as a control, receiving other treatment.

Cases Treated with Crofton Vaccine at R.S.P.C.A. Hospital, Putney

Symptoms evident for 1 to 14 days before commencement of treatment.

No. of cases	22
No. of cases destroyed	3
No. of cases recovered	19
Recovery	86 per cent.

Symptoms evident for 15 to 22 days or longer (having had no form of treatment).

No. of cases	7
No. of cases died	2
No. of cases destroyed	5
Fatalities	100 per cent.

Control cases receiving penicillin, sulphonamides, amphotropin, C.F.9, etc.

No. of cases	20
No. of cases recovered	7
No. of cases died	3
No. of cases destroyed	10
Recovery	35 per. cent.

The lower percentage of recoveries with vaccine treatment in this trial was partly due to poor home management. In many cases the disease was further advanced than the owners would admit, and low nutrition, rickets and worm infestation were common.

Average number of injections given was six.

Prophylaxis.—For over a year Mr. Orr has been using a mixed stock vaccine prepared for him by Dr. Crofton from cases of distemper and hard pad, as a means of inoculation.

During this period none of the puppies inoculated (two doses of vaccine were given) has shown symptoms of either disease.

On this test alone it is impossible to assess how valuable this prophylactic treatment may be, but Mr. Orr is firmly convinced of its usefulness and safety.

He has also used this stock vaccine on some cases under treatment, but results were not so spectacular as with autogenous vaccine.—Yours faithfully, Ruth McLelland, 6, Clarendon Drive, Putney, S.W.15. *September 3rd*, 1952.

* * * * *

REPORT OF THE COMMITTEE ON LICENCES

Sir,—Although agreeing with Major Kirk that there has been considerable abuse of the services of animal welfare society clinics and that local veterinary surgeons in private practice have suffered much inconvenience and irritation from their activities, we cannot agree with him that the future of private small-animal practice is so black as he would have us believe.

We all have welfare society clinics operating in the near vicinity of our places of practice, in most cases considerably nearer to us than has Major Kirk, yet we have ample work and busy practices.

We would add that several of these have been built up, and are still progressively expanding, during the last decade when the welfare societies have been so active in establishing new clinics.

We feel, therefore, that the public still requires and appreciates the private practitioner and that there is an adequate living still to be made from small-animal practice by those who offer a reasonable service at reasonable fees.—We are, yours faithfully, M. A. W. Bentley, London, S.W.10; W. B. Conn, Edgware; F. B. Halpin, Eltham; J. O. Joshua, Finchley; J. M. Wilson, Enfield.